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Indices of Potential Lead Hazard

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This review is concerned with the concentrations of lead in human whole blood, erythrocytes, plasma, serum, soft tissues, bone, and urine. The extent to which redistribution of some of the bound lead occurs is outlined. The effects of lead on enzyme activities and on the accumulation of metabolic intermediates in the blood and urine are described. A brief section deals with the range of signs and symptoms that can occur and differences seen between symptomatic children and adults.

The issue here is the assay for individual and group health hazards due to lead as assessed by biochemical methods on body fluids and tissues. These are "indices" of lead hazard but might also be considered "sentinels," in that they attempt to guard the gate appropriately, by allowing effects not particularly hazardous to pass while calling attention to more reasonable hazards. Some other terms used are also discussed. This range of effects partly overlaps the one for which the term "subclinical effects" (*1*) has been used. When some of the latter are detected, they are hard to relate in a proven manner, individually, to lead owing to their subjective and nonspecific natures.

As an early line of defense, therefore, we are dependent mainly upon assay for the lead concentration in body fluids, enzyme activities, the presence of abnormal concentrations of some metabolic intermediates, and if possible, the evidence of lead exposure. Signs and symptoms are, of course, considerably used in practice along with some of these assays.

The review is part of a consideration of vehicular lead emission as it contributes to the hazards of lead (*2–6*).

Terminology

The use of the term "normal" or "normal value" is often abused. For this reason, the distinctions between "normal," "reference" and "discrimination" or "decisive values" will be considered generally first, then specifically with regard to lead hazard. The general part of this section owes much to an editorial by Sunderman (*7*).

The usefulness of the term "normal value" varies. Its accuracy is compromised by a poor correlation between values in and outside the normal range with the presence or absence of observed signs and symptoms. This poor correlation can be due to the following somewhat overlapping causes: (a) the occurrence of transient and wide fluctuations in values unassociated with clinical change, for example, due to ingestion or inhalation of a substance followed by its relatively rapid uptake and clearance from the blood; (b) insufficient specificity of the test for the component being tested; (c) poor correlation between the value in the tested body fluid and the degree of involvement of receptor sites—structurally, quantitatively or over time; (d) better correlation with a subfraction of the fluid, for example, with plasma or serum, or with another body fluid, for example, spinal fluid, or with concentration in a particular organ; or (e) the requirement for a prolonged period before the symptoms become manifest.

Furthermore, there are three logical ambiguities in the use of the word "normal": (a) ambiguity in context—it connotes healthy in the clinical sense, Gaussian in the statistical sense, and either ideal, conventional, or habitual in the popular sense; (b) circular reasoning—a "normal" population is one which is, overall, free of a condition while the presence of differences from this group defines, overall, the presence of the condition; and (c) emotional connotations—what is "normal" is acceptable while the "abnormal" is hazardous or frankly harmful and should be corrected. It would be surprising, however, if the terms normal value and

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normal range were eliminated from use in the near future. An attempt is being made to encourage the use of more accurate terms.

The use of "reference values" and "reference intervals" is being fostered in Europe and North America. As an example, 97 and 100°F (36.1 and 37.8°C) might be reference values for two individuals or two groups without clinical evidence of disease, while this variation from 98.6°F (37°C) could be indicative of disease in others. The reference value is a function of the following: (a) the vitae of a reference population; (b) external and internal conditions of the collection of the specimen; (c) the analytic method used along with both its precision and accuracy; (d) the statistical tests used.

The terms "discrimination value" and "decisive value" are even more specific. These are claimed (*8*) to be the "best dividing lines between the 'normal' and the 'diseased' or between 'those who need not be investigated further' and 'those who do.' " It is often not possible to obtain this degree of specificity with a single test. However, two or more tests can frequently be used to distinguish between the groups of individuals that are "normal" and "diseased," or that "need not be investigated further" and "those who do."

Sunderman (*7*) lists the factors for the determination of a "discrimination value" as: (a) a clear definition of the disease, (b) clear demarcation of the stage to be specified by the test, (c) ability to detect a high proportion of true positives, (d) ability to eliminate other conditions (a low rate of false positives), (e) knowledge of the prevalence of the disease, and (f) consideration of the risk to the patient of a positive or negative misdiagnosis.

Specifically, when referring to a "normal" value for whole blood lead (PbB), it is more reasonable that a variety of reference values and reference intervals (or ranges) exist for the groups of infants, children, and adults of both sexes and also for different geographic populations and occupational groups.

Waldron (*9*) recognized the problem of referring to a normal value or a normal range for PbB and offered the following thoughts. "A more valid approach would be for each center to establish its own reference values taking into account such factors as diurnal and seasonal variations (*10–13*) and then to match the results against those for the appropriate reference population. The results could then be expressed either in terms of standard deviations from the mean or as an age-race-sex specific percentile such as Elveback (*14*) has described. Used in this way, blood lead estimations would alert the physician or environmental hygienist to an unrecognized source of contamination while at the same time serving as valuable corroborative evidence of excessive exposure in suspected cases of poisoning."

Chisolm (*15*) wrote, with regard to PbB determinations, that "difficulties arise . . . when one attempts to use this indicator [PbB] of *group* exposures as a diagnostic indicator of toxicity. There is a lively debate in the current literature concerning the choice and interpretation of various diagnostic tests for monitoring *individuals* who are at risk" (Chisolm's italics).

He discussed the use of new definitions of the terms "critical" and "subcritical effects," and "critical site." Critical effect and critical site were developed originally in the field of radiation biology; now, as agreed upon by a Subcommittee on the Toxicology of Metals (*16*), the term "critical effect" has been adapted "for metals such as lead . . .[as] . . . the most sensitive and specific biologic change beyond acceptable physiologic variation which is caused by the metal (*17*). Although different effects may be produced by a metal, the 'critical effect' is the *first* measurable adverse effect not necessarily the most serious one. 'Subcritical effects' are measurable biologic changes which do not impair cellular function." "Adverse" and "impair" appear to be equivalent. While neither the meaning of adverse nor the distinctions between impaired and not impaired are competely clear, Chisolm clearly means that there is an operational, if not an actual difference upon which we proceed. Unequivocal distinctions are difficult, if not impossible, to define and describe, for example, what we may consider serious or nonserious at one time can, with new data or an acceptable new interpretation, be accepted in an alternate manner. The last paragraph of this section also applies.

"The 'critical site' is the location in the body where the 'critical effect' occurs; it may be a system, organ, cell type or cell component. . . . This approach has advantages from the viewpoint of preventive medicine. Intervention on the basis of first or 'critical effects' at an early reversible stage should prevent the occurrence of later adverse effects that may have serious consequences from the clinical viewpoint." And then, specifically for inorganic lead, "derangement of hemoglobin synthesis in the erthyroid cells of the bone marrow is the 'critical effect' " (*16, 18*). . . . Decreased ALAD [δ-aminolevulinic acid dehydrase] activity, as measured *in vitro* in mature red cells in peripheral blood, is considered a 'subcritical effect' (*16*) though it is a highly useful indirect indicator of exposure (*19, 20*)."

In another vein, when it is determined that a PbB value for an individual or group is outside its reference interval, this concentration, alone, does not

specify either safety or hazard for the individual. For this reason we next would consider the enzyme assays and product accumulations as well as the signs and symptoms.

A variety of conditions can affect the values assayed. For example, most of the lead in blood is associated with the erthyrocytes. Because the proportion of erythrocytes in blood normally follows a complex curve from the prenatal period through puberty, a correction for hematocrit (Hct) was proposed. Thus, before birth and until about 1 mo of age the Hct is greater than in adults; it decreases below the adult male and female levels by 1–2 yr, and then rises slowly to the adult levels by the teen years (*21*). Hct also varies with several disease conditions. A decreased Hct is sometimes seen with evidence of moderate or higher levels of lead absorption. Correction for the Hct has been controversial (*22–28*) and has not been generally applied. It is discussed further in the next section. In some cases, for example at very high PbB concentrations when plasma lead is increased, or when studying effects directly on the erythrocytes, the correction would be appropriate in order to obtain the most accurate PbB values.

The "normal concentration range" for PbB in humans has been reported extensively. All determinations were not, obviously, made with the same degree of precision or accuracy, and much has been written about inter- and intralaboratory variations. Lauwerys et al. (*9*) summarized a study that involved 66 European laboratories in the analysis of lead, mercury, and cadmium, and referred to earlier comparative studies.*

They wrote, "the variability of the results is not attributable to the use of different analytical methods" [the three methods used most for the lead were flame and flameless atomic absorption spectrometry and colorimetry; two others were polarography and anodic stripping] "or difference in experience, because precise results were obtained by laboratories using different techniques and having different degrees of experience." However, only 42-65% of the laboratories were "precise," i.e., had a demonstrated "true" interlaboratory coefficient of variation (CV_0) of $\leq$ 10%. "Furthermore, . . . the laboratories that measure the metals with precision do not exhibit the same degree of accuracy. Heavy metal concentrations in blood and urine cannot therefore be directly compared when the measurements are performed by different laboratories, even when the respective precisions . . . are satisfactory." However, with an assured standardization technique, the results of different laboratories would be comparable. "It appears that systematic errors are responsible for the high interlaboratory variation. . . . Further standardization of their methods in order to reduce . . . systematic differences [are necessary]. However, meaningful relative comparison of different 'at-risk' groups remains possible when all the analyses . . . in biologic material are performed by the same [precise] laboratory."

Other than for the imprecise analyses, differences in accuracy may also be partly a function of the low concentration of lead and the ease of contamination during collection, transportation or assay. To protect against some of these problems, when a PbB concentration appears increased, a second specimen should be drawn and reanalyzed.

Last, it should be kept in mind that hazards are not only in the short range. These can become manifest: (a) at the time of assay or shortly thereafter, (b) in the future, either because of a prolonged time period before smaller detriments accumulate, (c) because cell differentiation has been affected and the sequelae become evident only after differentiation is well advanced, or (d) because of accumulation of lead in blood components and in soft and hard tissues, from all of which it is, at least potentially, available for release and uptake by sensitive tissues. Release from these sites is discussed below.

Lead in Blood and Urine, Tissues, and Bone

Whole Blood and Urine

Whole blood lead (PbB) concentration has been the most widely used test to monitor for a potential lead hazard. However, its diagnostic value at the borderline of hazardous conditions is quite limited. It has been found that PbB is more a measure of very recent exposure (*30–33*) than of total body burden, and most lead in the body is within the hard and soft tissues (*33–36*). Below are some of the best data for "normal" concentrations of PbB.

*The following samples went to all laboratories: four aliquots of human urine A, three of B; four aliquots of cow blood C, three of D; one sample of human blood E; three aliquots of aqueous solution 1, one of 2 (where 2 was an 0.1 dilution of 1). Fifty to 55 laboratories analyzed the blood and 33 to 34 the urines, for the lead. The mean PbB for human blood (1.5 l pooled from four donors who were not occupationally exposed to heavy metals) was 23.3 μg/100 ml (median of 18.1 and range of 1-115 μg/100 ml). The range was not as startlingly large in the other biologic and aqueous samples, 8.5- to 32-fold. The range includes both the "precise" and "imprecise" laboratories. When the "precise" laboratories only were considered, the interlaboratory coefficient of variation was still unacceptably large.

Goldwater and Hoover (*37*) reported normal concentrations of lead in whole blood (PbB) and urine (PbU on 150 ml of urine from a 24-hr collection) for over 800 specimens from 16 countries, including New Guinea (aborigine population) and the U. S. The subjects were mainly hospital employees or clinic patients of both sexes and were screened to eliminate those with unusual exposures to lead. Chemically clean glass or polyethylene bottles were used for the samples which were then mailed to a single laboratory in the U. S. for analysis by a standard dithizone method.

They defined "normal" as "the amount found in persons who have had no evident occupational, medicinal, or other unusual sources of exposure." The "normal range" found was about 5–40 μg/100 ml,† but the values obtained included 45, 50, 53, 60, and 100 (New York City) μg/100 ml with, in all likelihood, more values in the latter range. The distribution was skewed as seen by the low median (18 μg/100 ml) and mean (17 μg/100 ml). For about 95% of the tests the values were ≤40 μg/100 ml.

Goldwater and Hoover (*37*) referred to a comprehensive review of the world literature by Stopps, presented at a meeting (*38*) also referred to by Stopps, et al. (*39*) and reproduced partly elsewhere (*40*), which yielded similar as well as slightly higher values for PbB between 1941 and 1965 and higher values in some cases between 1931 and 1940 (Fig. 1). The assays of PbU are shown for comparison. McLaughlin and Stopps (*41*) have determined PbB and PbU, respectively, in 420 and 3819 employees of E. I. duPont de Nemours & Co. who were not working with lead products, at 23 locations in the U. S. and analyzed the data for nonsmokers vs. smokers. Mean PbB values were: nonsmokers, 19.1 μg/100 ml; cigarette smokers, 19.9 μg/100 ml; other smokers 17.3 μg/100 ml. The values were not significantly different. However, PbU was said to be significantly (very slightly) less in the nonsmokers than in the two other groups; 27.1 vs. 28.6 and 29.0

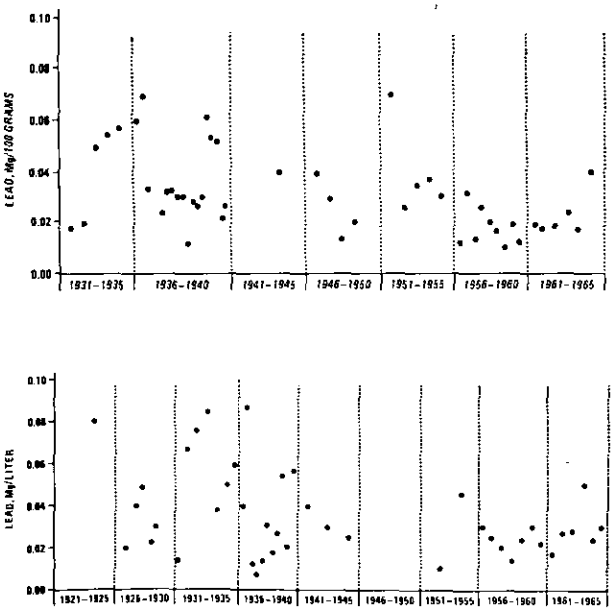

FIGURE 1. Concentrations of lead: (top) in whole blood lead (PbB), reported in studies from 1931 to 1965); (bottom) in urine (PbU), reported in studies from 1923 to 1965. Data from Stopps (*40*) with permission.

μg/100 ml, respectively. They quote several other studies of smokers and nonsmokers that had slightly different results. Thus, in one study, 21 μg/100 ml was found in smokers and 16 μg/100 ml in nonsmokers, while another found 16.4 and 16.3 μg/100 ml, respectively.

Butt et al. (*42*) reported the mean PbB value for 47 presumably normal hospital employees to be 17.7 ± 0.8 (SE) μg/100 ml.

Robinson et al. (*43*) reported blood lead data for 103 children of ages 5 hr to 13 yr, where there was normal temperature and no history of pica or previous lead poisoning or symptoms suggestive of lead poisoning. The values for nine neonates ranged from 7 to 28 μg/100 ml; for 28 infants up to 6 mo of age and including the neonates, 5-31 μg/100 ml (median, 15 μg/100 ml); for 75 children between 6 mo and 13 yr, 3-54 μg/100 ml (median, 27 μg/100 ml, with 90% of the values between 15 and 40 μg/100 ml). The difference between the values for infants less than 6 mo and children 6 mo to 13 yr was statistically significant. The 6 mo to 13 yr values were pooled for statistical analysis since there was no difference between those 6 mo to 4 yr and 4 yr to 13 yr. All values were shown on a scattergram.

Erythrocytes, Soft Tissues, and Bones

At least 90% of the lead in blood is generally associated (*24, 43, 44*) with the erythrocytes (PbE).

†There are three corrections in the Goldwater and Hoover report (*37*). The corrected values are given in the text and the justification here. (a) From the data, the normal range of PbB values is 5–40 rather than 15–40 μg/100 ml. (b) To be consistent with the paragraph directly above this in their report and also with two values given in their Summary and Conclusions, 95% of the blood lead values rather than 98% was meant. And most importantly, (c) the mean plus two standard deviations (2SD) would give 40 rather than 50 μg/100 ml as the value that includes 95% of the blood lead values; 3 SD would yield 99% of the values up to 50 μg/100 ml. The interpretation (c) is consistent with the paragraph above in their report where 95% of the PbU values are included in 2 SD (about 65 μg/100 ml). Dr. Goldwater concurs with these corrections (personal communication).

In *in vitro* tests, lead is transferred rather quickly from the plasma to erythrocytes (*45, 46*). At low PbB values and under some other conditions, as noted below, the plasma and serum contain a higher percentage or an additional amount of lead. There are at least two erthrocyte compartments for the lead, one associated with the membrane and the other within the cell (*46*). The lead is more easily displaced from the membrane and displaced with more difficulty from inside the cell (*46–48*). While some believe that the erthrocytes are mainly an inactive transport and storage site for the lead (*24, 27*), others suggest that uptake by the erythrocytes may not be quite so benign (*26*). Some of the difference of opinion, however, is due to definition.

Angle and McIntire (*23*) point out that, assuming 90% of PbB is in the erythrocytes, at a Hct of 45%, a 20 μg/100 ml PbB concentration would mean PbE was 40 μg/100 ml; at 35% Hct, PbE would be 85 μg/100 ml. Stretching this further (*26*), at a Hct of 45% and PbB of 40 μg/100 ml, PbE would be 85 μg/100 ml; at 30% Hct, it would be 160 μg/100 ml. Hct values of 25–30% have been reported for 42 children at PbB concentrations of 70–136 μg/100 ml (*24*). The higher concentrations in the erythrocytes, therefore, in the anemic condition, may represent an additional burden to normal development or functioning of the erythrocytes. Evidence to support the contention was presented (*23, 26*). However, the higher concentrations in the erythrocytes may not have a relationship to the effects of lead on the nervous system, kidney or other tissues (*25*). While the reductions of hemoglobin (Hb), Hct, and erythrocyte count can occur with lead absorption, they are not generally associated with the early stages of toxicity or with PbB values up to at least 50 μg/100 ml, unless there has been a previous chronic exposure at perhaps higher levels. The mechanisms by which the effects on erythrocytes come about are complex and include the inhibition of Hb synthesis (both the heme and globin moieties), abnormal erythrocyte production, and crenation and fragility of the erythrocytes leading to hemolysis. Basophilic stippling is well known in lead toxicity but it is also caused by many other conditions. The percentage of reticulocytes is sometimes increased (*28, 49–52*).

Maxfield et al. (*53*) found a slight increase of Hb, Hct, and the erythrocyte count in dogs fed lead acetate along with their diet. The lead acetate was fed at 100 ppm and also at 500 then 1000 ppm for 46 wk. They mention studies by Cantarow and Trumper (*54*) and Pecora et al. (*55*). [the latter quoted by Waldron (*56*)] that suggest that at low lead concentrations, the lead appears to stimulate erythropoiesis. The mechanism by which this occurs was suggested to be, perhaps, as secondary to either hypoxia or hemolysis. Maxfield et al. (*53*) also could not find interference with the recovery rates for Hb, Hct, or erythrocyte count in dogs after the dogs had been bled in two stages to produce 30–40% reductions of these three erythrocyte parameters. However, alternate results were obtained in the baboon by Kneip et al. (*57*) and in the rat by Gelman and Bus (*58*).

Rosen et al. (*24*) assayed Hct in 135 children with PbB of 20–136 μg/100 ml. In the two groups with PbB of 20–29 and 30–39 μg/100 ml, mean Hct was 36%; at 40–49 and 50–59 μg/ml it was 34%; at 60–69 μg/100 ml it was 33% . . . and at 100–136 μg/100 ml it was 26%.

Lead is also taken up by soft tissues and by bone. Much has been written about these sites of lead accumulation. Some recent studies of the concentrations attained in soft tissues and bone are by Gross et al. (*34*), Barry (*35*), and Poklis and Freimuth (*36*). The data were analyzed as a function of age, sex, occupation, and geographic location.

Rabinowitz et al. (*33*) studied lead under dynamic conditions at physiologic concentrations in five healthy men with the stable isotope, ^{204}Pb. The data obtained could be explained on the basis of a three-compartment model, though it is more likely that there are many compartments (pools). The first pool is the blood. It contained 1.7–2.0 mg of lead in the five subjects and had lifetimes of 27–40 (mean 35) days. This pool is in direct communication with ingested lead, PbU, and the second and third pools. Since the mean lifetime in pool one was 35 days and the mean lifetime of erythrocytes is 110–120 days, it is inferred that lead enters and leaves the erythrocytes several times during the erythrocyte lifetime. This study supports the finding of Butt et al. (*42*).

The second pool consists mainly of soft tissues. It contained 0.3–0.9 mg of lead and had lifetimes of 30–55 days in three individuals. It includes the hair, nails, sweat, and salivary, gastric, pancreatic, and biliary secretions. The total for these two pools is about 3 mg. This is less than the total of 10–30 mg found in soft tissue at autopsy by Schroeder and Tipton (*59*) and the 9.3 mg mean in nonoccupationally exposed men, the 15.7 mg in men with occupational exposure, and the 6.2 mg in women, found by Barry (*35*).

The third pool is mainly the skeleton and contains most of the lead in the body. Gross, et al. (*34*) estimated 200 mg of lead in the body, and Barry (*35*) reported mean values in men of 165 mg total lead without occupational exposure, 522 mg with occupational exposure, and 119 mg in women. On the whole, lead has a very long lifetime in the skeleton. Although the ^{204}Pb entered both the dense cortex

and spongy trabecular bone, a more rapid turnover occurs in the trabecular bone, as seen by the two- to threefold greater ratio of ^{204}Pb to total lead compared to the cortex.

On a comparative basis, Rabinowitz et al. (*33*) point out that their results in humans differ from those reported for the rat and hamster. When rats and hamsters inhaled radioactive lead, lead accumulated in the erythrocytes only to about half the extent, there was more found in the kidneys, and clearance from the blood was more rapid (*60*). In another study, rats were given a trace amount of ^{210}Pb in 25–250 μg of carrier by single intravenous injection, an amount which may have altered the normal handling of lead and also erythrocyte survival time. More lead then occurred in the feces and also transferred to the skeleton, while again it was cleared more rapidly from the blood than in humans (*61*, *62*).

There is great interest in conditions that affect release, redistribution and excretion of lead from erythrocytes, soft tissues and the bones. Under some circumstances, for example, satisfactory chelation therapy, beneficial results occur; in other circumstances, toxic episodes have either been reported or suggested.

The lead of erythrocytes occurs both within the cells and in association with the membrane in a ratio of 9:1 (*63*). Within the cell it is bound to hemoglobin, to a colorless component with molecular weight of about 10,000 and to other lower molecular weight components (*64*), perhaps phosphate (*45*), 2,3-diphosphoglycerate (*23*), and others (*27*). Human erythrocytes appear to be saturable with lead *in vitro* only at concentrations greater than 200 μg/100 ml PbB. Thus, only above this concentration did an increasing amount of lead remain in the plasma following centrifugation (*46*). Rosen et al. (*24*) found a similar relationship *in vivo* in children, in which case the plasma concentration remained constant up to a PbB value of 136 μg/100 ml. Higher PbB concentrations than these have been found in occupationally exposed workers, usually with little lead occurring in the plasma (65).

The incubation of rabbit erythrocytes with ^{203}Pb (*64*) showed that 94% of the isotope was taken up in 1 hr. This uptake was inhibited in a dose-dependent manner by nonradioactive lead and more so by Fe^{3+} as well as Fe^{2+} and Imferon, a parenteral Fe^{3+} preparation. Of the three ^{203}Pb components separable by Sephadex chromatography of hemolyzates—Hb, a component with molecular weight about 10,000 and a small molecular weight component—only ^{203}Pb in the smallest component decreased upon the addition of Fe. Incubation with Ca, Mg, Cu, Hg, Zn, Co, Ni, and Cr did not have the effect of Fe. The rabbits then received Fe in saline, *in vivo*, at 4 mg/kg or saline alone, in each case 1 hr before administration of ^{203}Pb. At 1 hr after FE, only 21.6% of ^{203}Pb was taken up by the erythrocytes, while after saline 95.6% was taken up. Various aspects of the roles of iron deficiency and iron deficiency anemia for the hazard of lead was discussed in the report.

Human erythrocytes equilibrated with lead for 6 or 24 hr lose lead, on addition of $CaNa_2EDTA$, more slowly than comparable erythrocytes equilibrated for only 15 min. It appears that the lead transfers from a more easily accessible or weakly bound site, to a site where it is less accessible or more tightly bound (*46*).

Rosen and Haymovits (*66*) collected erythrocytes from rats treated with lead and suspended these in solution with $CaCl_2$ at Ca^{2+} concentrations of 0, 2, 4, 6, and 8 mg/100 ml. The mixtures were incubated for 15–30 min. PbE decreased by 0, 0, 12, 24, and 40%, respectively, within the 15-min period. At preincubation PbE values of <50, 51–99, and 100–180 μg/100 ml, release of lead by Ca^{2+} was, respectively, negligible, 15%, and 38%, indicating that only above a threshold PbE concentration could lead be displaced (*67*).

Forced entry of Ca^{2+} into the erythrocytes was then achieved by addition of A23187, a compound that reacts with Ca^{2+} to form a lippophilic complex (*47*). At suboptimal Ca^{2+} concentrations, greater displacement of lead then occurred, though at optimal concentrations there was no further displacement. Rosen and Haymovits suggested that a larger displaceable fraction is on the surface of the erythrocytes while a smaller but also displaceable fraction was either within or below the membrane.

In six lead-intoxicated children, the concentration of Ca^{2+} in serum increased by 1.50 ± 0.2 (SE) mg/100 ml above the baseline during chelation therapy with $CaNa_2EDTA$. The increase closely paralleled the decrease of PbB. Furthermore, the subsequent decrease of Ca^{2+} in the serum to baseline coincided with the increase of PbB shortly after EDTA therapy was terminated. It was suggested that a portion of the EDTA effect in these children resulted from the Ca^{2+} that was coadministered with EDTA (*48*).

There have been many explanations offered concerning why one individual develops symptoms and another not, and what causes periodic exacerbations and then subsiding symptomology. These include: (a) the differential availabilities of "diffusable" lead vs. lead that is stored at more inaccessible sites, (b) recent high dose vs. more chronic exposure and accumulation, which may operate through the principles of differential accessibility, (c) an in-

crease in the absorption or reabsorption of lead from the gastrointestinal tract, (d) physiologic "trigger" mechanisms such as parathyroid, thyroid, or calcium effects (among others), that could mobilize stored lead particularly in bone, but perhaps also in erythrocytes or soft tissues, (e) inadvertent mobilization of lead by transient acidosis, febrile conditions, dietary components, and perhaps some drugs, and (f) an increase in the concentration of plasma or serum lead. Goyer and Mahaffey (*68*) wrote of differential effects sometimes seen in association with age, season, calcium and phosphorus imbalances, iron deficiency, protein, vitamin D, ascorbic acid, nicotinic acid, alcohol, other metals, coexistent diseases, and with the formation of intracellular inclusion bodies. Sorell et al. (*69*) also discussed some of these.

Early techniques for therapy attempted to immobilize the lead by using conditions that would make it either less soluble or more easily deposited in bone (*70*). More frequently and at present, the removal of diffusable lead or stored lead is attempted under conditions that try to avoid the development or exacerbation of symptoms.

Since it has become somewhat common to treat children with moderately high PbB concentrations with chelating agents in an attempt to avoid apparently more subtle lead hazards, reasons for a general avoidance of pharmacologic de-leading have also been presented. In its place, there would be an aggressive lead screening and treatment program, conducted by a nurse practitioner in a family-focused neighborhood health center (*71*). In this study, it eventually became necessary to treat six of the children with $CaNa_2EDTA$ because of a breakdown of the lead hazard abatement program, pica by the children, ages less than 3 yr and a rapidly increasing PbB concentration within 2–3 mo (from 40–50 μg/100 ml to 60–80 μg/100 ml). However, 31 children in this study were not treated by chelation. Rather, PbB was decreased over a 7-mo period by natural clearance mechanisms and with the aid of elimination of lead from the environment and behavior techniques. The peak mean PbB concentration in the untreated group was 55 μg/100 ml, and the highest single value was 76 μg/100 ml. These 31 children were chosen for intensive follow-up that lasted 18 mo because their PbB concentrations were more than 40 μg/100 ml for three successive determinations. The argument is properly made, that the medical need to bring about a rapid change in the children and also to avoid undue stresses on overworked medical and paramedical facilities must be balanced against the psychic trauma often associated with hospitalization of young children and adverse reactions than can occur with the chelation therapy.

In some attempts aimed at mobilization and removal of the lead, PbB increased but the urinary or fecal excretion of lead did not. When a considerably increased excretion of lead occurs a portion of the lead should derive from the more mobile fraction of bone, since, especially in adults, the erythrocytes and soft tissues contain only a very small portion of the total body lead.

Some of the therapeutic regimens used to increase the excretion of lead, or at the least remove some "diffusable" lead, have included: low calcium, high phosphorus diet (*72*), low calcium diet with or without the coadministration of NH_4Cl (*72, 73*) or H_3PO_4 (*73*), Na_2HPO_4 (*74, 75*), sodium or potassium iodide (*76, 77*), sodium citrate (*78*), dimercaprol (2,3-dimercaptopropanol, BAL) (*79–81*), penicillamine (*81–83*), and edathamil (calcium versenate, $CaNa_2EDTA$) (*81–85*).

Castellino and Aloj (*86*) and Hammond (*87*) studied the removal of lead from body sites by $CaNa_2EDTA$. Castellino and Aloj (*86*) used rats. They gave a dose of lead and showed that when EDTA administration began 1 day later and continued daily, a rapid then a slower phase of lead elimination from whole blood, erythrocytes, liver, kidneys, lungs, and heart occurred. They suggested that the EDTA removes extracellular lead (the rapid phase), shifting the equilibrium concentrations between the cellular and extracellular compartments, which is necessary for further elimination (the slower phase). Urinary excretion of the lead increased while fecal excretion decreased. When EDTA was begun 7 days after the lead, only small quantities of lead were now released from the same tissues; the urinary excretion increased while fecal excretion remained the same. Lead in the femur, as a representative of bone, was not released in either experiment.

Hammond (*87*) used rats and rabbits. He first treated the rats with $CaNa_2EDTA$ on days 4, 17, 34, and 60 after the lead. The concentrations of lead that remained were then assayed in aliquots of nitric acid digests of pooled bones, pooled soft tissues, erythrocytes and pooled urine and feces. The amount of lead released, as in the previous study (*86*), decreased as the span increased between lead and EDTA administration. Although the mobile bone pool of lead was small when compared to the total of the bone pool, this fraction was still much greater than the mobile soft tissue pool. Because of this relationship, bone was, quantitatively, the main site for the lead released. It was suggested that the analysis of femur only, by Castellino and Aloj (*86*),

did not represent a proper sampling of the types and states of bone tissue. In the erythrocytes, the lead concentration stabilized within 17 days and then remained nearly constant.

Hammond (*87*) found that at various times after EDTA administration there does not appear to be a simple, constant relationship between the amount of lead in soft tissues and the amount released by EDTA. There was also not a constant proportionality between the removal of lead from the soft tissues and from the skeleton. Rabbits responded similarly.

Although bone may account quantitatively for most body lead released by EDTA and both the mobile and less mobile fractions of lead in bone are still important with regard to secondary redistributions and potential hazards, it is presumably either, or both, the "diffusable" and the more stable lead in some of the soft tissues that accounts for the symptoms of lead toxicity.

Jugo et al. (*88*) found that $CaNa_2EDTA$ administered to 15-day and 18-wk-old rats after lead resulted in a lesser excretion of the lead in young rats. The same effect occurred with BAL as the chelating agent, and it was independent of the dose, route and frequency of administration of the lead and also of the molar ratio of chelating agent:metal. They proposed that there may be a lower free lead or active lead concentration in the young rat due to greater binding strengths of the lead for tissue ligands and cited supportive data from the work of others.

In the statement on increased lead absorption and lead poisoning in young children by the Center for Disease Control (*89*) it is noted that "children who require chelation therapy will also require long-term medical surveillance and care. 'Rebound' of blood lead levels resulting from release of lead from tissue pools after an apparently successful course of chelation therapy should be anticipated."

The amount of "chelatable lead" in a carefully controlled 24-hr urine sample can sometimes be a better indicator of the "internal dose" of lead than PbB (*15*). Provocative chelation, also referred to as an EDTA mobilization test, has been used as a diagnostic test in asymptomatic patients. In this case there should be particular attention to the possible induction of symptoms (*89*).

An alteration in bone or teeth of binding, more permanent deposition and the redistribution of lead would be expected in association with a variety of normal and clinical conditions, for example: (a) during early development, (b) continued growth at the epiphyses until maturity, (c) conditions that involve bone remodeling or resorption and (d) during pregnancy (*69, 91–93*). At these times, the lead may be more mobile than in quiescent periods.

Analyses of the lead in different bones and in different parts of the same bones (*34, 35*) and the metabolic, compartmental studies of Rabinowitz et al. (*33, 90*) show that bone is heterogeneous with regard to the uptake and metabolism of lead. There are both rather mobile and relatively stable compartments. EDTA *in vivo*, for example, will mobilize some of the lead. It was suggested many years ago that lead appears to follow calcium with regard to the skeletal system and that factors that affect the state of bones would be expected to affect its lead content (*94*).

Recently, Rosen and co-workers have been studying the effects of hormones and ions known to affect bone. They have looked for the ability to alter the lead content of bone in organ culture and have worked with fetal rat bones. Pregnant rats were given the normal isotope of Pb in drinking water throughout gestation and then a dose of ^{210}Pb a day before sacrifice. Rosen and Wexler (*95*) investigated the effects of parathyroid hormone (PTH), calcitonin (CT), inorganic phosphate (P_i), Ca, and Mg on fetal radii and ulnae over a 48-hr period.

They found that at a low concentration of Ca (0.7*mM*), resorption occurred, and some of the formerly deposited ^{210}Pb was released. Addition of PTH to the medium did not cause any further release and assay for total Pb in the control and PTH-addition preparations showed similar amounts of Pb. Heat treatment of the bones to stop the metabolic activity of their cellular fraction stopped the release of the ^{210}Pb suggesting, though not proving, that physicochemical exchange alone was not involved. A high Ca concentration (2.8*mM*), however, inhibited the release of ^{210}Pb due to PTH. With an intermediate amount of Ca (1.4*mM*), the decrease of ^{210}Pb was proportional to that of the total Pb, indicating that a selective exchange of the more recently acquired ^{210}Pb was not involved. Finally, the release of Ca from the bones approximately followed the time course of release of Pb.

An increase of P_i concentration (2*mM*) inhibited the release of ^{210}Pb in both the control and PTH-addition preparations, but more so in the latter. Increasing P_i further to 4*mM* inhibited the release from each to an equal extent.

At a low concentration of Mg (0.4*mM*), PTH-induced release was again inhibited more so than in the control (non-PTH) preparation. At a high Mg concentration (1.6*mM*), no effect was detected.

CT at a normal P_i concentration (1*mM*), reduced the amount of ^{210}Pb released to below that of the control preparation. Increasing P_i to 2 and 4*mM* further inhibited release and in an approximately additive manner with the CT. Assay for the total Pb

also showed that more Pb remained when CT and the extra P_i were present.

In these experiments, the mobile Pb compartment was about 20% of the total of ^{210}Pb incorporated. It was stated, as an as yet unpublished observation, that both EDTA and increased culture times of 72 and 120 hr showed the mobile compartment to be two- to threefold greater, and closer to that found for Ca by others. They also tried to relate their findings to *in vivo* situations by reference to the literature and to some of their own studies.

It is quite clear from the studies sampled in this section and those cited elsewhere (*96*) that the erythrocytes, soft tissues, and bones can be active with regard to the turnover of lead within them due to a variety of conditions. Some sites are quite stable. However, many will turn over at least a portion of their lead in response to normal, inadvertent, or purposeful influences.

Plasma and Serum

As noted, usually 90% or more of PbB is associated with the erythrocytes. Accurate assay of the plasma (PbP) and serum lead (PbS) have been more difficult because of the usual methodologic difficulties and low concentrations.

However, there have been studies and comments that indicate the PbP and PbS can be increased at times. It has been assayed in greater amounts, for example, in association with toxic symptoms (*65, 97*) and with the use of $CaNa_2EDTA$ (*86, 98*) for the purpose of de-leading individuals. However, the PbE and noncell concentrations have also been increased without apparent symptomology, and vice versa. Toxicity has been attributed to a variety of free and bound forms of the lead (on proteins or smaller chelating molecules, inorganic molecules, etc.) which would then be variably available for transport through membranes.

PbP and PbS are in either rapid or more slowly achieved equilibrium with essentially all body storage sites (*99*). It is in closest proximity to both the erythrocytes and to the most vascular tissues, less so with immature and mature bone. The extra lead appearing in the noncellular fraction of blood could, therefore, have several sources: (a) erythrocyte damage and release, (b) nondamaging release from erythrocyte-bound sites, (c) release from soft or hard tissues, (d) the occurrence of a defect in the erythrocyte or tissue uptake of either newly absorbed lead or of the reuptake of lead that has been released from binding sites, or perhaps (e) all of these, depending upon the particular conditions.

In vitro studies concerning uptake and release with isolated erythrocytes and in bone organ culture were reviewed in the previous section.

Bambach et al. (*44*) referred to nine reports in which lead in the blood was suggested to be mainly in the cells, four in which there was found a more equal distribution with the plasma, and only one in which it was the plasma that had most lead. In their study, they found at least 90% in the cells of rabbit blood. This was the case even with PbB values up to 140 and 290 μg/100 ml. They used the colorimetric method with dithizone for analysis.

Robinson et al. (*43*) reported PbP for 82 children, apparently of ages 5 hr to 13 yr, with normal temperature and no history of pica, previous lead poisoning, or symptoms suggestive of lead poisoning. They used a dithizone method. In 53 lead was not detected, in 22 it was <9 μg/100 ml, and in seven it was >9 μg/100 ml. Since hemolysis was said to occur to some extent in the seven latter cases, this could account for at least a part of the lead.

Butt et al. (*42*) reported serum lead concentrations for three groups in the Los Angeles area. The analysis was by emission spectrometry and it was part of a 15 element survey. With 122 blood donors, the mean serum concentration was 3.9 ± 0.2 (SE) μg/100 ml; with 96 patients hospitalized for elective surgery or generally for a variety of degenerative diseases, the mean was 2.7 ± 0.5 μg/100 ml; and with 48 normal employees of the hospital, the mean was 2.8 ± 0.3 μg/100 ml. In the second group, for 7, 10, 9, 36, and 27 individuals, respectively, newborn, ages 5–19, 20–29, 30–49, and 50–79, the mean serum lead values were 6.5, 1.3, 2.6, 2.4, and 3.3 μg/100 ml. Standard errors were not given for these data. It was said that the "metal serum levels were rather uniform in all classifications such as age, race, sex, or length of residence in the Los Angeles area."

Farrelly and Pybus (*100*) analyzed serum lead by atomic absorption spectrometry in a portion of the individuals they studied who had either no unusual exposure to lead or had varying exposures. They were primarily interested in the packed red cell concentrations. They stated that "even in cases of lead toxicity, we find the serum lead to be less than 5 μg/100 ml." These data are not presented.

Rosen et al. (*24, 48*) determined plasma lead concentrations by flameless atomic absorption spectrometry for 165 children including: 13 newborns, 14 and 17 children, respectively, with "normal" PbB values of 20–29 or 30–39 μg/100 ml; 8-24 children in succeeding 10 μg/100 ml PbB range groups up to 90–99 μg/100 ml; 10 children in the 100–136 μg/100 ml range; and 17 children with sickle cell disease, who had PbB concentrations of 18–136 μg/100 ml. The plasma lead concentration for each group was between 1 and 7 μg/100 ml, with the mean concentrations 3.0–3.3 μg/100 ml.

Repetitive daily measurements of PbP prior to treatment also showed concentrations around 3.0 μg/100 ml (personal communication). The two groups with the highest PbB values (100–136 μg/100 ml and the children with sickle cell disease, 18–136 μg/100 ml) included some children with encephalopathy (*48*). However, the relationship of the time of blood sampling to the occurrence of encephalopathic symptoms was not mentioned, and PbP was not determined during the chelation treatment (Rosen, personal communication).

It is not uncommon to see asymptomatic children in the presence of relatively high blood lead concentrations. In discussing the report by Rosen et al. (*24*), Chisolm (*25*) remarks "whether plasma lead concentration is measurably higher in symptomatic cases remains obscure." In one of the reports (*48*) some cases of encephalopathy are referred to, in the groups with PbB concentrations of 100–136 and 18–136 μg/100 ml, while in their other report (*24*) this notation was not made. Fluctuations of PbP on an hourly basis cannot be ruled out and a potential effect of the chelation therapy was not sought. Indeed, Castellino and Aloj (*86*) state, "in the blood, lead was mainly bound to the cells and more than 10% was found in the plasma only at the beginning of the treatment with chelant" ($CaNa_2EDTA$, in the rat, no mention of associated symptomology).

Bessman and Layne (*98*) administered $CaNa_2EDTA$ to two adults and three children and determined either PbB or PbE along with PbP in each case by means of a dithizone method. The analyses were done prior to treatment and at 2, 4, and 8 hr after treatment (Table 1). Prior to treatment, PbB was above normal in patient 2 (61 μg/100 ml) and PbE and PbP were above normal in patient 3 (275 and 63 μg/100 ml, respectively). The treatment appeared to increase the lead concentration for each parameter, but to different degrees and over different time courses. The PbP concentration increased in each case, to a considerable extent in some of the patients (patient 2, 27 and 48 μg/100 ml; patient 3, 123, 80, and 55 μg/100 ml; patient 4, 139 μg/100 ml). Bessman and Layne did not mention whether or not there was associated symptomology in these specific cases. An earlier report (*84*) described the use of $CaNa_2EDTA$ during the acute stage of encephalopathy.

Table 1. Effect of $CaNa_2EDTA$ on plasma, erythrocyte, and whole blood lead concentrations.[a]

Patient	Age, yr	Blood fraction	Lead concn, μg/100 ml			
				After treatment		
			Before treatment	2 hr	4 hr	8 hr
1	Adult	Erythrocytes	15	—	23	3
		Plasma	2	—	17	19
2	Adult	Whole blood	61	—	70	100
		Plasma	0	—	27	48
3	3	Erythrocytes	275	186	68	34
		Plasma	63	123	80	55
4	4	Erythrocytes	12	—	109	—
		Plasma	0	—	139	—
5	5	Whole blood	7	12	10	12
		Plasma	2	22	17	12

[a]Data of Bessman and Layne (*98*).

An early and extensive report by Smith et al. (*97*) sought a relationship between PbS and the degree of symptomology observed in those who had been exposed industrially (24%), from paint (2%), by the administration of lead colloid for cancer chemotherapy (56%), from x-ray usage (1%) and in an unknown manner (18%). Despite important methodologic criticisms, including some aspects of the assay for lead and the subjective nature of many of the signs and symptoms, the study is worth noting for comparison with other reports. The data should be considered either semiquantitatively (Table 2) or qualitatively (Table 3).

A dithizone method was used for lead assay. A concentration of 10 μg/100 ml [the data were converted from mg/10 g (taking 1 g = 1 ml) to be consistent with most other reports] was said to be the level at which the data should be accepted "qualitatively rather than quantitatively." The study was done in the same laboratories as the one by Willoughby and Wilkins (*101*), a variant of whose method was utilized. Willoughby and Wilkins (*101*) also state that the error in the recovery of lead added to cow blood and analyzed for PbB, was ≤ 10 μg/100 ml for 41 of 42 samples when the lead was added in quantities of 20–100 μg/100 ml. This amount, then, is the lower limit for the analysis though not for detection. An apparently rigorous procedure was followed to avoid lead contamination of glassware and hypodermic needles.

There was awareness of the need to "minimize any changes in equilibrium of the serum and cell lead" from their description of defibrination and separation. They also referred to several reports to support the use of serum instead of plasma on the basis of trying to avoid lead shifts from erythrocytes to the serum. Bambach et al. (*44*) reported a few years later than PbP and PbS concentrations did not significantly differ despite the use of heparin, citrate, oxalate or cooling in an ice bath for 1 min to prevent clotting, or in serum, for rabbits who were fed lead acetate. They suggested that this "indicates that all these results can probably be compared to those obtained by workers using the serum-clot separation."

Smith et al. (*97*) cited many references to the

fragility, alteration, and destruction of erythrocytes sometimes seen in lead toxicity. If hemolysis occurred in some samples, one would expect that it would have been mentioned. However, there is no mention of hemolysis or the exclusion of samples, which is an improbable situation considering the large number of samples and analyses. Some loss of lead from the erythrocytes to the serum, cannot be excluded. For the two groups of "normal" individuals, they note that "in no instance was lead detected in the serum . . . by the analytical method employed. This has been substantiated by Willoughby and Wilkins in a recent series of patients without signs or symptoms of plumbism." However, Willoughby and Wilkins (*101*) remark "of the 58 separated specimens, only six contained slightly positive amounts of lead in the serum fraction, this fraction from all other samples being found lead-free. However, no significance can be attached to these few positive serum lead values because each was less than 0.001 mg [10 μg/100 ml], which is within the limits of experimental error."

Finally, Smith et al. (*97*) state that "at the approach of death there are indications of a general breakdown of the body's biological and physiological equilibriums accompanied by a flood of lead into the peripheral circulation which is neither eliminated nor detoxified in the manner of the normally functioning defense system. It is shown in Table 13 [of Smith et al.] that in cases of latent, mild, or inactive plumbism, the distribution of the lead in the serum, cells + fibrin fraction and whole blood tends to equalize each other at, or shortly before death."

For 41 patients (Table 2) who had clearly present signs and symptoms, PbS was 20-150 μg/100 ml (mean, 58 μg/100 ml). For 25 of the 41 patients (60%), PbS was from 50-100% that found for PbB.

For 35 patients with either acute or active chronic lead toxicity, but in whom the clinical signs and symptoms were weak or temporarily absent, PbS was 10-40 μg/100 ml (mean, 20 μg/100 ml). For eight of the 35 patients (22%), PbS was 50–100% of that found for PbB.

For both of the above groups, the concentrations of lead in the cells + fibrin and PbB fractions were greater than for the comparable analyses of the two "normal" groups shown in Tables 4 and 5 of Smith et al.

For 18 individuals who were mainly 4 mo or more past either an acute toxic reaction or the active phase of chronic toxicity (in one case 2 mo), lead was below the assayable level in the serum. For these individuals also, the lead in the cells + fibrin and PbB were greater than for "normal" groups (Smith et al., Tables 4 and 5).

For the 36 "normal" individuals (doctors, nurses, technicians, secretaries and one artist) and 47 patients hospitalized for nonlead-related reasons, lead was below the assayable level in serum. The mean concentrations of lead in the cells + fibrin (62 and 67 μg/100 ml) and PbB (36 and 33 μg/100 ml) were higher than would be ob-

Table 2. Lead concentration in the serum, cells + fibrin and whole blood of five groups of lead-exposed and "normal" individuals.[a]

Smith et al. Table No.	No.; types of individuals	Concentration, μg/100 ml		
		Serum	Cells + fibrin	Whole blood
9	41; acute and active phase of chronic toxicity, with clearly present signs and symptoms	20–150 (mean = 58)	50–510 (mean = 152)[b]	50–280 (mean = 109)[b]
10	35; same as Table 9, but weak or temporarily absent signs and symptoms	10–40 (mean = 20)	30–370 (mean = 127)[c]	20–180 (mean = 73)[c]
11	18; mainly 4 mo or more after acute or active chronic episode	Nil	140–640 (mean = 250)[d]	70–220 (mean = 117)[d]
4	36; "normals"	Nil	10–110 (mean = 62)	10–50 (mean = 36)
5	47; hospitalized for nonlead reasons	Nil	20–130 (mean = 67)	10–60 (mean = 33)

[a]Data of Smith et al. (*97*).

[b]The 41st patient, not included in these values, showed the following concentrations: serum, 30 μg/100 ml; cells + filbrin, 730 μg/100 ml; whole blood, 410 μg/100 ml.

[c]The 35th patient, not included in these values, showed the following concentrations: serum, 20 μg/100 ml; cells + fibrin, 2,290 μg/100 ml; whole blood, 830 μg/100 ml.

[d]The 18th patient, not included in these values, showed the following concentrations: serum, nil; cells + fibrin, 1,110 μg/100 ml; whole blood, 640 μg/100 ml. This individual was studied 2 mo after the toxic episode.

tained today, but approximately that obtained at the time (Fig. 1). The concentrations found for the "normals" of their Table 4 were said to be essentially independent of sex, age (adults), climatic changes, daily fatigue, violent exercise, meals, menstruation and ovulation. Today, with more sensitive methods, we know that meals and perhaps some other of these factors would affect the concentrations of at least PbB.

It is seen in Table 3 that the patients were rated for their degree of lead symptomology. This was done using a ranking of the signs and symptoms collected from nine reports. Smith et al. compared these changes with change in concentration of PbS at the time of the ratings. Some of the patients were studied for up to 150 days. Three patients died. Qualitatively, there appears to be an association between the direction of PbS change and direction of change of symptomology. The data cannot be considered quantitatively because of the coarseness of the symptom ratings and because many PbS concentrations were near 10 μg/100 ml, their assayable limit.

In a subsequent report (*102*) they summarized the concentrations of lead found in serum, cells + fibrin and whole blood under normal conditions and in the "active phase of acute or chronic toxicity," "latent phase" and "inactive chronic phase." They also presented the concentrations for an additional case studied, along with the leucocyte and erthyrocyte concentrations of lead.

Table 3. Relationship between change of lead concentration in serum and change of clinical status.[a]

Patient NO.[b]	Sequence of serum concentrations, μg/100 ml	Sequence of clinical conditions
1	20, 60	Poor, died
2	30, 20, nil	Poor, improved, well
3	nil, 20	Fair, symptoms after acidification therapy
4	nil, 40, 50, nil	Good, fair, fair, well
5	nil, 30, 20	Fair, poor, died
6	nil, 20, 30, 50, 90, 30, nil	Good, fair, worse, bad, bad, improved, well
7	10, 50, nil	Good, fair, well
8	10, 50, 40, 30, nil, nil, 30, nil	Good, poor, improved, better, well, fair, poor, well
9	nil, 50, 20, nil	Good, poor, improved, well
10	nil, 40, 20	Good, fair, fair
11	10, 20	Fair, fair
12	10, nil, nil	Fair, good, well
13	30, 20, nil	Fair, good, good
14	30, 50, nil, nil	Fair, poor, improved, well
15	nil, 30	Poor, died
16	nil, 20, 20, 50, 50	Fair, fair, poor, poor, worse
17	30, nil, nil, nil	Fair, fair, good, well

[a]Data of Smith et al. (*97*).

[b]In the report the patients are identified by initials, sex and type of lead exposure.

More recently, McRoberts (*65*) was the appointed doctor for a lead battery factory in the U. K. He tried to avoid some of the problems of Smith et al. (*97*). He drew 6–8 ml of heparinized blood in lead-free syringes and sent these to the National Occupational Hygiene Service for analysis. Other clinical chemistry and hematology assays were done locally. Of 81 initial blood samples from 21 men, seven were assayed directly for PbB and the remainder were centrifuged for analysis of the plasma and cell fractions. Hemolysis occurred in 20 samples and, therefore, only PbB concentrations were reported for these (the sum of the two fractions). In all, there were 54 PbP analyses for 18 men. Six duplicate random samples were assayed satisfactorily as a check on precision. Since a dithizone method was used, the differences between the very low PbP values may be in question.

Five of the 18 men studied were categorized as having had some of the signs and symptoms of lead toxicity while, except for transient anemia in some cases, 13 did not.

The first of the five individuals was occupationally exposed to lead for about a month. PbB and Hb concentrations over the next 2 mo were: PbB, 510, 790, and 535 μg/100 ml; four Hb values were between 11.7 and 13.2 mg/100 ml. PbU and urine coproporphyrin (CPU) assays at the end of 2 mo were low normal. Over this period, several physicians could not detect clinical signs or symptoms. However, because of the worker's very high PbB concentrations, the decision was made to attempt deleading.

On the morning of hospitalization 3 wk later and the following morning (Table 4), PbP concentrations were, respectively, 16 and 71 μg/100 ml, and PbB concentrations 313 and 295 μg/100 ml, all quite high. Occasional stipple cells were noted, and a slight tremor was detected at this time, though other clinical and laboratory tests were normal. A test dose of $CaNa_2EDTA$, less than recommended for deleading, was administered intravenously over 2 hr. At 4:30 p.m., PbP and PbB were still high, 59 and 297 μg/100 ml, respectively. In the evening, there was an encephalopathic reaction which included cardiac and respiratory arrest. Chisolm warned against this hazard when either too low or insufficiently frequent doses of $CaNa_2EDTA$ are given (*103*). PbU tests over several days showed that 2.6 mg of extra lead was excreted. For 2 days and at a month after, PbP concentrations were now, respectively, 6, 3, and 10 μg/100 ml while PbB decreased but was still in the hazardous range at the end of this time.

A second worker became anemic within 3 mo at the factory and was then suspended because of low

Hb. Twelve PbP concentrations over 2 mo (Table 4) varied from 3 to 8 μg/100 ml, with four interspersed values of 12–22 μg/100 ml. Over this period, PbB increased to a plateau and then declined. Hb remained low for a month and then began to rise slowly. Over the first month of suspension he complained of tiredness and intermittent, vague pains in the muscles of the arms and legs. Examination didn't reveal abnormalities.

The third worker was suspended after 7 days because of the low Hb (Table 4). This did not improve over a month with oral iron therapy. PbP values were 10, 15, and 4 μg/100 ml over a month while PbB values were, respectively, 117, 66, and 22 μg/100 ml. Only the persistent anemia was present as a sign, and because of this, worker 3 is discussed further below.

The fourth worker was away from work because

Table 4. Lead concentrations in the plasma, cells, and whole blood of five lead battery workers with signs and symptoms of lead toxicity.[a]

Worker	Date	Hb. g/100 ml	Pb, μg/100 ml			Time
			Plasma	Cells	Whole blood	
1	5/27/69	14.0	16	621	313	11:45 a.m.
	5/28/69	—	71	527	295	9:00 a.m.
	5/28/69	—	$CaNa_2EDTA$, *i.v. administration*			Noon-2 p.m.
	5/28/69	—	59	544	297	4:30 p.m.
	5/28/69	—	Encephalopathic episode			7:25 p.m.
	5/29/69	—	6	583	283	9:00 a.m.
	5/30/69	—	3	500	241	9:00 a.m.
	6/23/69	—	10	272	141	
2	7/18/69	10.8	4	138	87	
	7/19/69	11.4	3	155	84	
	7/21/69	11.4	22	230	120	
	7/24/69	11.5	4	253	106	
	7/26/69	11.4	3	253	106	
	7/28/69	11.8	3	280	115	
	7/29/69	11.9	2	280	108	
	7/31/69	11.4	17	250	118	
	8/02/69	11.4	16	211	102	
	8/06/69	11.8	8	223	101	
	8/09/69	11.0	Hemolyzed		87	
	8/13/69	11.2	12	203	94	
	8/22/69	12.0	5	156	78	
	9/19/69	12.6	Hemolyzed		66	
3	12/08/69	11.2	10	279	117	
	12/16/69	11.6	15	116	66	
	1/07/70	11.3	4	42	22	
4	1/27/70	15.2	16	114	85	
	2/24/70	14.9	5	126	67	
5	2/05/70	12.6	Hemolyzed		88	
	2/07 to 2/23/70		Away from work			
	2/24/70	13.1	6	920	415	
	3/03/70	12.4	Hemolyzed		381	
	3/10/70	13.8	Hemolyzed		435	
	3/13/70		Stopped work			
	3/17/70	13.5	12	1034	480	
	3/19/70	10.9	Hemolyzed		515	

[a]Data of McRoberts (65). Workers identified by initials in original report.

of "influenza" symptoms (Table 4). Two blood samples a month apart showed, respectively, PbP, 16 and 5 μg/100 ml; PbB, 85 and 67 μg/100 ml; Hb was normal. He complained of malaise, weakness and general aches and pains.

The last worker had a moderate degree of anemia (Table 4). However 67% (four out of six) of his blood samples hemolyzed. This was the highest proportion observed for the 18 workmen studied. PbP concentrations 3 wk apart were 6 and 12 μg/100 ml and the PbB values, respectively, 415 and 480 μg/100 ml. Other PbB concentrations are shown in Table 4. He complained of tiredness and irritability.

For these five "symptomatic" individuals, then, the PbP values for 10 out of 25 samples were 12–71 μg/100 ml. Except for the single PbP assay after $CaNa_2EDTA$, nine of the increased plasma concentrations were not associated with pharmacologic intervention. In one of the nine, there was said to be fever at the time. PbB for 31 samples was 22–515 μg/100 ml. In addition, there were two PbP concentrations of 10 μg/100 ml, with corresponding PbB values of 141 and 117 μg/100 ml. The first worker also had PbB concentrations of 510, 535, and 790 μg/100 ml.

The following, now, are the data for the 13 men who were said to be nonsymptomatic. The PbP for 28 samples was 1-7 μg/100 ml and PbB for 43 samples was 33-173 μg/100 ml, with outlying values of 18 and 250 μg/100 ml. There was also one PbP value of 10 μg/100 ml, with the corresponding PbB value of 82 μg/100 ml.

Closer examination of the data shows that either "symptomatic" worker 3 could have been moved to the nonsymptomatic group because he had only anemia for the 1 mo that he was followed, or four workers classified as "nonsymptomatic" could have been considered symptomatic on the basis of their "transient" anemia. Another three "nonsymptomatic" workers were not studied long enough to say whether their borderline anemia was transient or persistent. The choice can be made more easily to transfer worker 3 to the nonsymptomatic group because transient versus persistent anemia is a quantitative issue and his relatively low PbB concentrations supports the assignment. While these considerations call attention to a weak point, it does not eliminate the pattern found.

Therefore, although the increase of PbP in the group of five (or four) workers occurred in only about one-third of the samples, the most striking difference between the groups was that this difference occurred. McRoberts (*65*) suggested that a PbP of about 10 μg/100 ml might be the dividing area between asymptomatic and symptomatic states. He wrote: "most recent work has been concerned with the establishment of biological criteria to confirm the diagnosis of lead poisoning, and this is of the utmost importance in the preventive aspects of industrial medicine, but there appears to be a place for continuing research into the biochemical or biophysical trigger mechanism which induces a state of intoxication. Further investigation into the part played by 'lead integration' [McRobert's term for the binding of lead in erythrocytes and perhaps in soft tissue cells] and the reasons for its failure may afford an opportunity to develop new therapeutic measures." And unfortunately, "although the estimation of the plasma lead fraction might give presumptive evidence of imminent intoxication, technical difficulties preclude its use as a routine test."

Tissue Effects

Enzymes, Intermediates, and End-Products

Assay for enzyme activity, intermediates, and end-products, incorporation of precursor molecules, concentration of compounds along shunt pathways or otherwise abnormal products, and rate of excretion or secretion are used extensively to assess subclinical and clinical reactions. The aim here is to preclude or reduce illness in the following ways: (a) detection of reversible stages of abnormal tissue function, when these are present, in order to avoid the irreversible effects; (b) identification of hyperreactive individuals or population groups, whether the hyperreactivity is due to genetic, nutrition, occupational, regional or other factors; (c) determination of hazardous combinations of exposure, where neither alone may be of particular hazard.

Tissues that are affected by lead absorption include the hemopoietic system, central and peripheral nervous systems, kidney, gastrointestinal tract, skeletal muscle, liver, immune system, reproductive system, and cardiac muscle. Most biochemical and physiologic tests so far have assayed aspects of hemopoiesis and also the mature erythrocyte. There are many fewer biochemical studies for the nervous system and the kidney, and improved tests are greatly needed for these. Tests that operate in the region of the critical and subclinical effects are, ideally, what are desired. Effects below these may be indicative of exposure, though not particularly of hazard, while effects at and above these levels should be responded to with more concern.

Dose-Effect and Dose-Response Relations

Dose-effect and dose-response relationships were discussed recently with regard to the clinical and epidemiologic aspects of lead absorption (*15, 16, 104, 105*). When the law of biologic random variation applies, the dose-effect and dose-response curves have the same general form.

Dose-effect assesses quantitative change in enzyme activity, concentration of a metabolite or specific function for an individual. It is the most used relationship for a pharmacologic or toxicologic reaction. Test results that indicate an abnormally high or low value, either without concurrent symptoms or with symptomology, respectively, have been designated false-positives and false-negatives. Dose-effect does not initially recognize the "reactors" and "nonreactors" who are two or three standard deviations from the mean. Individuals can vary also with regard to the threshold, slope, and maximum effect of their effect curves. The threshold or no-effect level is, actually, a practical no-effect level, below which the difference from zero cannot be distinguished for a variety of reasons.

Dose-effect assumes either that we know the concentration of a toxic agent at the site of action, usually not the case, or that, for example, blood or urine concentration is an accurate indicator of the "internal dose" at the reactive site. Occurrence of transient, rather wide variations of PbB were already discussed. Chisolm states "though it is possible that this assumption may be of some use under carefully controlled steady-state conditions, it is increasingly evident that it is not valid in the highly unstable epidemiologic circumstances under which childhood plumbism actually occurs (*15*)."

Dose-response, also called dose-percent, relates to the proportion of a group or population that responds to a defined extent for a particular effect. Dose-response is useful in clinical and epidemiologic investigations. It identifies the reactors and nonreactors. Accurate dose-response curves are dependent upon the size and selection of the group.

Zielhuis (*105*) suggests that "because literature data do not yet cover large groups of exposed subjects, one should not try to approach zero-% too closely, e.g. 0.1, 1.0%; one should use about 5%. Thus, one can estimate a practical non-response level, i.e. not inducing a specific intensity of a specific parameter [more than] in 5% of the subjects." The finding of a value more than the mean + 2 or 3 SD for an individual would classify the individual as a reactor, subject to confirmation.

Biochemical tests for lead absorption and toxicity have been reviewed often (*15, 32, 49, 89, 96, 99, 104–109*). Some of this information will be presented along with newer information, as it relates particularly to effects with the low lead exposures.

Hemopoiesis and Mature Erythrocytes

Inhibitory effects on Hb synthesis in the erythroid cells of the bone marrow is a "critical effect" of lead, since this is a reversible adverse effect at the lowest doses so far determined, preceding significant renal injury and clinically evident neurologic manifestations (*15, 16, 18*). The immediate results of the interference are the accumulation of a variety of intermediates. Further effects at higher concentrations include those on the degree of progression of the stages of maturation of the red cells, effects on globin synthesis, and effects on the morphology and stability of the cells (*32, 49, 89, 96, 104, 105, 109*). These effects can have several concomitants: (a) functional deficits are proposed to ensue in primary or secondary relationship to the extent of the effects, (b) the effects may be a predictor of related or unrelated effects occurring in other tissues, and (c) at the earlier stages, it may be primarily an appropriate sentinel, prior to the occurrence of more hazardous responses.

The sequence of conversion from the reaction of glycine with succinylcoenzyme A to the completed heme molecule has long been a subject of study and was outlined recently (*110–112*). The points of known and suspected interference by lead are noted (*99*). Some of the intermediates are, sequentially: δ-aminolevulinic acid (ALA, the aliphatic addition product of glycine + succinylcoenzyme A), porphobilinogen (PBG, a pyrrole formed by cyclization and loss of H_2O), uroporphyrinogin III (UPG, believed to be the first cyclized tetrapyrrole in heme synthesis), coproporphyrinogen-III (CPG), protoporphyrinogen IX (PPG), and protoporhyrin IX (PP). The final step is insertion of Fe^{2+}. The tetrapyrrole "ogen" compounds are all colorless and nonaromatic and are rather easily oxidized to the aromatic porphyrins.

The usual compounds and media that have been assayed for an effect of lead absorption are: δ-aminolevulinic acid dehydrase (ALAD, the enzyme that converts ALA to PBG, and which is present in erythrocytes and in other tissues), δ-aminolevulinic acid (ALA, in urine); coproporphyrin III (CP, the oxidized product from CPG, excreted in urine); and protoporphyrin IX (present in erythrocytes and usually assayed in the whole blood). The other compounds and enzymes in the sequence have been studied with regard to genetic

porphyrias and photosensitization reactions, less so with regard to lead hazard.

Zielhuis' Overview. Zielhuis (*105*) reviewed and suggested the following PbB concentrations as the non-response (< 5%) levels for the parameters indicated. In some cases, the PbB concentration for greater degrees of effect were also estimated.

ALAD. No-response (no inhibition) level, about 10 μg Pb/100 ml; >40% inhibition in adults, 15-20 μg/100 ml and in chilren, 5-10 μg/100 ml; >70% inhibition in adults, 25-30 μg/100 ml and in children, 20-25 μg/100 ml.

PPE (FEP). Erythrocyte protoporphyrin and free erythrocyte protoporphyrin are used interchangeably here. No-response in adult males, 25-30 μg Pb/100 ml and in adult females and children, 20-25 μg/100 ml.

ALAU. No-response for urinary excretion of ALA > 5 mg/l, 30-40 μg Pb/100 ml; > 10 mg/l, 40-50 μg/100 ml.

CPU. Urinary excretion of CP is about the same as for ALA, but there appears to be some evidence that the PbB concentrations will be lower for women.

Hematologic Effects. Preliminary evidence is cited for inhibition of erythrocyte membrane Na^+K^+ATPase and for the reduction of erythrocyte concentration of GSH. The slope of the dose-effect curve for Na^+K^+ ATPase is small. For Na^+K^+ATPase activity < 40 μmole P_i/hr/mg tyrosine, 20-29 μg Pb/100 ml; for GSH < 58 μg/100 ml of erythrocytes, 25-30 μg/100 ml. Anemia (decreased Hb) does not occur in otherwise healthy adults below 70-80 μg/100 ml, and in socioeconomically poor children below 40-50 μg/100 ml.

Other Effects. No-response levels for other effects are also given. Other biochemical parameters (a mixed, nonhemologic group that includes effects on 5-hydroxyindolacetic acid, uric acid, bilirubin, serum glutamic oxaloacetic transaminase and pyruvate) the no-response level is said to be at least 50-60 μg Pb/100 ml. For subjective symptoms the no-response level is > 50 μg Pb/100 ml. No-response level for encephalopathy is >> 80 μg Pb/100 ml in adults and > 50-60 μg/100 ml in children. For minimal brain dysfunction, the no-response level is > 40-50 μg Pb/100 ml; for peripheral neuropathy, > 40 μg/100 ml; for late clinical sequelae (also mixed, nonhemologic groups of many types of diseases), at least 40–60 μg Pb/100 ml.

Zielhuis (*105*) stresses that the words "effect" and "response" are neutral in that they do not imply a deleterious effect on health, nor does it necessarily correspond to "unacceptability." Thus, while minimal to moderate inhibition of ALAD is acceptable in the absence of other lead hazard findings, some of the others would clearly not be acceptable and others would constitute cause for concern and protective action. This is not in distinction to Chisolm's description of the "critical effect" and "critical site" concepts, since the critical effect is the first measurable adverse effect, not necessarily the most serious one (*15*).

ALA, PBG, UP, and CP. The concentrations of PBG in urine and UP (uroporphyrin) in blood and urine are only slightly affected, if at all, in lead poisoning (*49*). The excretion of ALA in urine with some studies in serum, and excretion of CP in urine (though the latter is present in much lower concentration in blood but much higher concentration in feces), have been among the earliest and longest studied parameters of heme synthesis. Thus, a statement by 18 individuals (*106*) divided lead poisoning into three stages above the normal state and presented what they agreed upon as appropriate concentrations of ALAU (δ-aminolevulinic acid in urine) and CPU (coproporphyrin in urine), PbU, and PbB. For the normal situation and the "acceptable," "excessive," and "dangerous" categories of exposure, their appropriate ALAU concentrations were, respectively, <6, 6–20, 20–40 and > 40 mg/l; CPU concentrations were, respectively, < 150, 150–500, 500–1500 and > 1500 μg/l.

Browder et al. (*32*) state that lead poisoning should be considered when the following concentrations are found: ALAU, 5 mg/24 hr in children and 10-20 mg/l for industrial workers (*113*); CPU > 150 μg/24 hr in children and > 200 μg/24 hr in adults (*114*). Baloh (*99*) pointed out that the tests for ALAU and CPU in children are subject to high rates of false positive and false negative determinations and, for this reason, are much more useful in industry where PbB may be above 70–80 μg/100 ml. Among the data reviewed, Baloh quoted the extensive findings of Stanković (*115*) in industrial workers. Stanković (*115*) reported the following concentrations for the normal, "slight," "moderate" and "severe" categories respectively: ALAU, < 4.5, <10, >10, >20 mg/l; for CPU, <90, <180, >180 and >700 μg/l. In small children (*116*), the upper normal for CPU was 75 μg/24 hr and for adults (*117, 118*), 250 μg/24 hr. Baloh suggested that because of the considerable variability of urine output, a better value of upper normal for both children and adults is, perhaps, 200 μg/l (*119*). Chisolm (*15*) also presented data for ALAU in children and expressed it in units of mg/m²/24 hr. Most of the data in this report were recalculated from previous work.

Benson et al. (*50*) determined PbB, ALAU, and CPU changes in men after first starting work at a lead pigment factory and at weekly intervals for up

to 12 wk. Their findings were compared with those of Tola et al. (*19*), who also studied initial exposures. Hb remained at >13 mg/100 ml and there were no clinical symptoms. The preemployment PbB concentration was about 22 μg/100 ml. It rose by 1 wk and reached 60 μg/100 ml by 3 wk and then stabilized. The changes in ALAU and CPU correlated well with one another and for most subjects, ALAU and CPU did not increase in the first 2 wk. Then there was a variable response. In a group of nine, the increase occurred over a period of 3-4 wk followed by variable increases afterward. In a group of six, there was a muted response, with small change for both parameters over the entire time period. For two individuals, there was an exaggerated response by 2 weeks. There were some striking differences between individuals, which was felt to be the major finding made. It was suggested that different monitoring methods might be appropriate for the initial period of exposure as compared to later, when the individuals become more stabilized.

ALAU is one of the most specific indicators of lead toxicity, particularly in children. Only acute alcoholic intoxication and the genetic condition, acute intermittent porphyria, cause significant elevations of ALAU. CPU is less specific. It can be increased in rheumatic fever, poliomyelitis, infectious mononucleosis, alcohol intoxication, cirrhosis of the liver, acute toxic hepatitis, acute intermittent porphyria and iron deficiency anemia (*32, 99*).

ALAD and EP. ALAD is the second enzyme, after δ-aminolevulinic acid synthase, in the synthesis of porphyrins from glycine and succinylcoenzyme A. It catalyzes the synthesis of PBG. When inhibited, δ-aminolevulinic acid should accumulate if the enzyme is not in excess. There are many reports which indicate that erythrocyte ALAD inhibition is very sensitive to lead concentration, even down to a PbB of 10–15 μg/100 ml and sometimes less (*99, 104, 120*). The enzyme is activated (*120*) by low concentrations of Hg^{2+}, Cd^{2+}, and EDTA and inhibited by higher concentrations of each of these. It is activated by the sulfhydryl compounds glutathione, cysteine, dithiothreitol, and mercaptoethanol as well as by Zn^{2+}. Activation by the sulfhydryl compounds and by Zn^{2+} are additive, suggesting that activation occurs by different mechanisms. Zn^{2+} counteracts both the activation and inhibition caused by EDTA. EDTA and other chelating agents should be avoided *in vivo* if ALAD is to be assayed and fresh or cold-stored blood should be used for the assay.

The enzyme activity can be determined directly as PBG synthesis or as the ratio of activated to the nonactivated preparation. Genetic factors accounted for no more than a twofold variation in ALAD in this study (*120*). Elsewhere it has been shown that the enzyme can also be activated by heating to 60°C for 5 min (*121*), that genetic factors may contribute a rather large effect at low concentrations of lead (*122*), that acute alcohol ingestion can inhibit the enzyme activity but that activity is restored as the blood alcohol concentration declines (*123*) and that the enzyme from *Rhodopseudomonas spheroides* is inhibited by protoporphyrin and hemin (*124*).

ALAD was assayed in blood, brain, and liver of rats that received lead from birth, first via milk from lactating rats fed lead acetate in their feed, and then also from the feed, itself. By 30–40 days, blood and brain ALAD were inhibited by 80–90% and liver ALAD by 66% (*125*). In 22-day-old male rats exposed to three doses of lead via the maternal milk, ALAD was inhibited in a dose-dependent manner in erythrocytes and in the cerebellum. It was inhibited only at the highest dose in the telencephalon and brainstem. Sobotka et al. (*124*) suggested that the enzyme activity in these three brain regions might be due to residual blood, though little blood should have been present (*126*). Secchi et al. (*127*) showed that human liver ALAD is inhibited by lead. Wada et al. (*128*) demonstrated reduction of activity of ALAD and heme synthase (ferrochelase), and a reduced incorporation of ^{14}C-glycine into the heme and globin of erythroid cells of the bone marrow in workers that had PbB concentrations of 40–90 μg/100 ml.

The relevance of the ALAD test with blood was questioned on several bases: (a) the enzyme appears to be vestigial and plays no known role in the mature erythrocyte, (b) an excess of the enzyme is present, and (c) no decrement in biologic function is known at the lower end of the scale to relate to the decreased values (*104*). The test detects both acute and chronic lead poisoning and there is no lag, *in vivo*, in inhibition of ALAD after exposure to the lead. The test is not positive for iron-deficiency states (*129*). It was suggested that the test can be used at the lower end of the inhibition curve, at least as a sensitive indicator of apparent exposure to lead, though apparently not with regard to hazard.

The history of the clinical relevance of protoporphyrin (*130*) and porphyrins in general (*131*) is long. However it has mainly been in the last few years that this factor, in two major public health problem areas—lead hazard and iron-deficiency anemia—has received more than a minor amount of attention in comparison with the genetic porphyrias. Iron-deficiency conditions have been subdivided into those due to: insufficient iron intake, malabsorp-

tion, impaired transport, and impaired utilization of the iron (*130*). Other disorders can also occasionally account for the accumulation of protoporphyrin in the erythrocyte. These are: hemolytic anemia, sideroblastic anemia, anemia associated with chronic disease, and secondary polycythemia. The free erythrocyte protoporphyrin test (FEP) has also been suggested as an aid for differentiating the mild anemia and microcytosis seen in alpha and beta thalassemia trait due to iron deficiency anemia (*131*).

More than 95% of the prophyrin that accumulates in the erythrocyte following absorption of lead and in the genetic condition, erythropoietic protoporphyria (EPP), is protoporphyrin IX (EP) (*132*). This is also the case in iron deficiency anemia (*133*). In both lead absorption and iron deficiency, it is the Zn chelate of protoporphyrin that accumulates (*133*, *134*) while in EPP it is mainly the free, basic form of protoporphyrin (*134*). The Zn chelate is bound to the heme site on hemoglobin, while the basic form is bound to nonheme sites (*134*). Acidic organic solvents are used to extract "free" erythrocyte prophyrins (free of the Zn), while the intact Zn chelate can be extracted with acetone, ethanol or the detergent Ammonyx-LO (*134*). Some of the quantitative tests used for protoporphyrin assay extract nearly all of the porphyrins, while a few extract much smaller quantities. Tests that extract small amounts are often very sensitive to modifying conditions of many types and, therefore, these latter cannot usually be counted upon.

Photosensitivity is usually not a complication following lead absorption despite the presence of protoporphyrin in the erythrocytes to a concentration that can be equal to or greater than that found in EPP. In lead absorption, the erythrocyte Zn protoporphyrin concentration declines only slightly, if at all, as the erythrocytes age. In EPP, however, the protoporphyrin declines rapidly as the erythrocytes age (a lifetime about 3 days). Incubation of the erythrocytes in plasma showed rapid diffusion of EP into the plasma in the case of EPP but not after lead absorption. *In vivo*, plasma EP was also elevated in EPP but not in lead absorption. These findings were used to explain the migration of protoporphyrin into cutaneous tissue in EPP, which then results in photosensitivity. This does not occur in the case of lead absorption (*135*).

A variety of tests were developed for the assay of EP (these tests are usually referred to as EP, FEP, and occasionally PP or PPE; (*99*, *104*, *133*, *136–139*). Contrary to the ALAD assay, for which there is no time lag and which can detect acute exposure to lead, FEP change occurs in the bone marrow. The cells are released to the peripheral circulation as they mature and, thus, there is a lag in the increase of peripheral blood FEP. Because FEP stays with the erythrocytes through their lifetime, its assay detects a chronic effect.

Sassa, et al. (*136*) showed a plot of FEP concentrations for children at PbB concentrations of 20–80 μg/100 ml and obtained a correlation coefficient of 0.72, which is close to that obtained by others. When they replotted the data utilizing only children who were known to have essentially constant PbB for at least 3 mo, the correlation coefficient increased to 0.91. They also assayed FEP along with ALAD and uroporphyrinogin synthase (URO-S; for other of their studies) in nine pair of twins. Monozygosity was established by extensive blood group testing for four pair (*136*). The concentrations for twin pairs, were close in most cases for each of the parameters, suggesting a genetic component, as well, in the control of the amount of enzyme activity. There was a two- to threefold spread of values for FEP, fourfold for ALAD, and threefold for URO-S.

Penny et al. (*161*) compared PbB, ALAD, and FEP in eight pairs of monozygotic and seven of dizygotic twins. The intraclass correlation coefficient differences between mono- and dizygotic twins were significant for PbB ($p < 0.01$) and for FEP ($p < 0.05$). The difference for ALAD was not significant.

Landrigan et al. (*141*) studied children aged 1–9 yr at various distances from a lead smelter. PbB concentrations were remarkably high. Within 1.6 km of the smelter, 99% of 172 of the children studied had PbB $\geq$40 μg/100 ml and 22% had $\geq$80 μg/100 ml; PbB, as expected, decreased with increasing distance from the smelter. The usual logarithmic increase of FEP with increasing PbB concentration, as obtained by others, was also obtained here. Roels et al. (*142*) studied 143 children ages 10–15 yr as well as adult men and women near a lead smelter and in a rural area. In this study, PbB varied from 5 to 41 μg/100 ml for all of the children. They found that the inhibition of ALAD was most sensitive, to changes of PbB, FEP was almost as sensitive and there was a considerably lesser response for ALAU. The dose-response relationships obtained for the children were as follows (for each PbB group vs. the percent of ALAD and FEP values, respectively, of greater than the mean value $\pm$ 2 SD): for 59 children with PbB $<$10 μg/100 ml, ALAD 3.4% and FEP 1.7%; for 41 with 10–19.9 μg/100 ml, 7.3% and 9.8%; for 24 with 20–29.9 μg/100 ml, 67% and 46%; for 17 with 30–39.9 μg/100 ml, 100% and 88%; for two with 40–49.9 μg/100 ml, 100% and 100%. They found, in addition, that children were most sensitive to FEP alteration, women

less so, and men least sensitive.

Klein et al. (143) presented data for 1523 analyses on children who were all presumably under 5 yr of age. They reported the dose-response data for the percent of children in each combination of five PbB and two FEP groups: 490 children with PbB < 30 μg/100 ml and FEP >60 μg/100 ml, 8% or FEP >190 μg/100 ml, 0.2%; 562 children with PbB 30–39 μg/100 ml and FEP >60 μg/100 ml, 22% or FEP >190 μg/100 ml, 1.0%; 267 with PbB 40–49 μg/100 ml and FEP >60 μg/100 ml, 45% or FEP >190 μg/100 ml, 4.5%; 109 with PbB 50–59 μg/100 ml and FEP >60 μg/100 ml, 54% and FEP >190 μg/100 ml, 16.5%; 95 with PbB >60 μg/100 ml and FEP >60 μg/100 ml, 72% or FEP >190 μg/100 ml, 26.3%.

Reigert and Whitlock (*144*) conducted a time-course analysis for 349 children with regard to PbB and FEP concentrations. They confirmed the finding of Sassa et al. (*136*) that change in FEP represents a long-term and relatively stable effect. They wrote: "Short- and long-term comparison of the variations with time of whole blood lead and free erythrocyte protoporphyrins (FEP) suggests that changes in FEP are slow and predictable whereas blood lead changes are quite unpredictable. However, when FEP suggests a different clinical category from blood lead, the blood lead is likely to change in the direction predicted by the FEP. Comparison of FEP to blood lead at first contact in 349 children with mild elevation of blood lead reliably predicted which children would still have elevated blood lead six weeks later and which would fall or be normal. The observation of long-term follow-up in four groups of children with various combinations of FEP and blood lead indicated that the follow-up blood lead could be predicted to change in the direction indicated by the FEP measurement."

Chisolm (*15*) found that FEP is a sensitive indicator of the "internal dose" of lead as long as iron deficiency and its concomitant anemia were mathematically corrected by a determination of Hct or Hb. "In children with hematocrit ≥36% and PbB <30 μg/100 ml, we have found that erythrocyte protoporphyrin levels are low and independent of PbB concentration . . . ≥90% of children with PbB ≥50 μg/100 ml are positive reactors and that 50% response rate is associated with PbB in the 40–50 μg/100 ml range (*15*)." Elsewhere (*89*) it is stated that "significant numbers of children with blood lead levels of 30–39 μg/100 ml have shown evidence of metabolic impairment as detected by EP testing."

The values obtained in the FEP test are dependent upon the method used. Some standardization is now coming into this area. The FEP test is positive (+3 SD above the mean) when it is >60 μg/100 ml by the ethyl acetate, acetic acid, HCl extraction method (*89*). Piomelli (*138*) gives the equivalent values in other units: >5.3 μg/g Hb and >160 μg/100 ml of packed erythrocytes.

The Center for Disease Control published recommendations (*89*) for: (a) screening for lead absorption in children, (b) diagnostic tests after screening if this is indicated, (c) pediatric management where absorption or poisoning is found and also with regard to preventive retesting of "normals," (d) hazard control, (e) education of parents of children who are at risk, and (f) the reporting of detected cases of lead absorption or poisoning. They defined undue or increased lead absorption as: "confirmed [two successive determinations] blood lead levels 30–79 μg/100 ml or an EP [FEP] level of 60–189 μg/100 ml except where the elevated EP level is caused by iron deficiency." They defined lead poisoning in children as the existence of (a) a confirmed blood lead equal to or greater than 80 μg/100 ml whole blood with or without symptoms, (b) EP level equal to or greater than 190 μg/100 ml whole blood with or without symptoms, (c) confirmed blood lead 50–79 μg/100 ml with compatible symptoms which cannot be explained otherwise or with associated abnormal EP, ALAD, ALAU, or CPU levels or abnormal calcium disodium EDTA mobilization tests, or (d) EP level of 110-189 μg/100 ml with compatible symptoms which cannot be explained otherwise. Toxicity, overall, with respect to effects of lead in children, includes subclinical manifestations of biochemical derangements (such as increased EP) as well as overt clinical manifestations (such as encephalopathy, Fanconi syndrome, etc.)

Table 5 depicts their grouping of the four classes from normal to the extremely elevated state with regard to the lead and EP values. For example, it is stated that "although the EP standard is based on tests involving several tens of thousands of children, this experience is more limited than that associated with blood lead. There may be need for revision of these standards when increased experience with EP measurements becomes available." And, "the suggested guidelines refer to the interpretation of screening results, but the final diagnosis and disposition rest on a more complete medical and laboratory examination of the individual child." Additional diagnostic tests and pediatric management are discussed. The report gives an indication of the thinking applied to differential diagnosis and relative risks for lead hazard.

The PbB and EP concentrations of each Class are shown in Table 5. In Table 6, class I is subdivided into classes Ia and Ib. Class Ia includes

Table 5. Classification of normal and elevated concentrations of lead and erythrocyte protoporphyrin in whole blood.[a]

	Class I normal	Class II minimally elevated	Class III moderately elevated	Class IV extremely elevated
Pb, μg/100 ml	≤ 29	30–49	50–79	≥ 80
EP, μg/100 ml	≤ 59	60–109	110–189	≥ 190

[a]Data of Center for Disease Control (*89*).

Table 6. Classification of lead toxicity in children based upon Pb and EP Blood concentrations.[a]

	Class[b]			
Pb level, μg/100 ml	EP ≤59 μg	EP 60–109 μg	EP 110–189 μg	EP ≥ 190μg
≤29	I	Ia	Ia	EPP+
30–40	↓ Ib	II	↑ III	↑ IV
50–79	*	↓ II	III	↑ IV
≥ 80	*	*	*	IV

[a]Data of Center for Disease Control (*89*).

[b]EPP+-Erythropoietic protoporphyria; (*) = combination of results is not generally observed in practice; when blood lead is repeated, the results will generally indicate contamination of the first specimen; ↓ denotes downgrading of the estimate of risk of lead intoxication suggested by blood lead, altered on the basis of the EP results; ↑ denotes upgrading of the estimate of risk of lead intoxication suggested by blood lead, altered on the basis of the EP results.

children with an apparent effect on EP due to the lead, but with a transient or error-caused low lead value. In some cases the increase of EP will be due to iron deficiency anemia. This can be confirmed as described (*145*) and recovery from both the anemia and the increased ED by successful uptake and utilization of an administered iron preparation. EP can increase to as high as the 110–189 μg/100 ml level in iron deficiency anemia. It must also be kept in mind that both iron deficiency anemia and increased lead absorption may coexist.

Category Ib represents a possible downgrading of the relevance to lead toxicity since it may be that there was a transient increase of PbB or a contaminated sample. Class II is possibly downgraded in relevance when the lead is 50–79 μg/100 ml for the same reason. Repeated or further diagnostic tests are advisable.

Class III and Class IV are potentially upgraded in relevance when the PbB is <49 μg/100 ml and <79 μg/100 ml, respectively. This is so since the PbB determination may be abnormally low for the reasons stated above. Reassay or further diagnostic tests are in order.

The following combination is pathognomic for lead poisoning: (a) decreased ALAD in circulating erythrocytes, (b) increased ALAU, (c) increased CPU, and (d) increased FEP (*16*), though they may not all occur in a specific case.

If EP is ≥190 μg/100 ml and PbB is ≤29 μg/100 ml, this often indicates erythropoietic protoporphyria.

Recent EP Associations. Increased FEP, sometimes > 190 μg/100 ml, is now being seen in a small number of children other than in relation to iron deficiency, lead exposure, or the genetic condition erythropoietic protoporphyria (Chisolm, Reigart, personal communications). These may reflect other genetic (*136, 140*), nutritional, or otherwise environmental (*69, 146*) differences in iron absorption, transport, or utilization (*130*) or, perhaps other mechanisms, for example, affecting the biosynthesis or utilization of porphyrins more directly. An association is also being observed between another particular genetic aberration, sickle cell disease (SS), double hemoglobin heterozygosity (SC), and possibly sickle cell trait (SA) and an increase of FEP (Chisolm, personal communication). There are at least 16 cases of an association in children of increased PbB with peripheral neuropathy, a rare symptom in children (*147–149*), and among these there are six cases of SS and one of SC disease (*147–151*). The remaining nine cases of peripheral neuropathy were reported between 1922 and 1952 and the molecular basis of the hemoglobinopathies was first reported in the late 1940's. Possible causes for the associations are discussed (*147, 148*). Further, individuals with the hemoglobinopathies generally survive to adulthood. An association between some adult cases of hemoglobinopathy and some of the indices of lead hazard should occur.

Other Tissues

Lead is known to affect a variety of other tissues and, while the clinical and some physiologic aspects have been described, investigations at the biochemical level are less advanced than for hemopoiesis. Much is available, though, that cannot be reviewed here. The kidney and the nervous system have received considerable attention. The following reviews are some that are useful (*1, 16, 32, 96, 152–154*). A few recent references will introduce some aspects of the actions of lead on the tissues: liver (*155*), kidney (*156–158*), and nervous system (*126, 159–165*).

Clinical Sequelae of Lead Toxicity

The clinical diagnosis of toxicity due to lead is not always easy. It is made partly on the basis of subjective and objective symptoms, a variety of signs,

biochemical analyses, accumulation or excretion of metabolic products, and evidence of lead exposure. Individuals may be asymptomatic or symptomatic, manifesting a wide variety of symptoms, and be in varying states of hazard (*32*). Goyer and Rhyne (*153*) point out that "the onset of lead toxicity, even acute toxicity, is not a sharply defined event. Rather, it involves a continuum of change from normalcy to ill-health."

Smith, et al. (*97*) ranked the many signs and symptoms of lead toxicity in adults. He added the caveat, "It should be remembered that all of these symptoms will never be found in any single case and frequently a patient is presented for observation or treatment whose past history to lead exposure would cause one to expect symptoms conforming to group II or III [greater effects] when only those of group I [lesser effect] can be demonstrated." Lane et al. (*166*) gave the following list for adults of mild symptoms and signs: tiredness, lassitude, constipation, slight abdominal discomfort or pain, anorexia, altered sleep, irritability, anemia, pallor, and less frequently diarrhea and nausea. The presence of a blue line in the gums and of a metallic taste are useful indicators of increased lead absorption. Severe symptoms and signs include severe intermittent abdominal pain (colic), reduction of muscle power—for example wrist drop, muscle tenderness, paresthesia, and other symptoms or signs of neuropathy or encephalopathy. Dagg et al. (*167*) gave a shorter list for adults, in descending order of frequency of the presenting symptom: abdominal pain, constipation, vomiting, nonabdominal pain, asthenia, paresthesia, psychological symptoms and diarrhea.

The signs and symptoms in children are somewhat different than in adults. For example, peripheral neuropathy is more common in adults, while encephalopathy is much more common in children (*153*). The following are seen in children (*89*); irritability, vomiting, abdominal pain, ataxia, anorexia, behavioral changes, speech disturbances, seizures, intercurrent fever and dehydration.

The symptoms in descending order of frequency in children as listed by Sachs et al. (*82*) are as follows: drowsiness, irritability, vomiting, gastrointestinal symptoms, ataxia, stupor and fatigue.

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